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Chemistry of Carcinogenic Metals

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The periodic distribution of known and suspected carcinogenic metal ions is described, and the chemical behavior of various types of metal ions is explained in terms of the general theory of hard and soft acids and bases. The chelate effect is elucidated, and the relatively high stability of metal chelates in very dilute solutions is discussed. The concepts employed for the chelate effect are extended to explain the high stabilities of macrocyclic and cryptate complexes.

Procedures for the use of equilibrium data to determine the speciation of metal ions and complexes under varying solution conditions are described. Methods for assessing the interferences by hydrogen ion, competing metal ions, hydrolysis, and precipitation are explained, and are applied to systems containing iron(III) chelates of fourteen chelating agents designed for effective binding of the ferric ion. The donor groups available for the building up of multidentate ligands are presented, and the ways in which they may be combined to achieve high affinity and selectivity for certain types of metal ions are explained.

Carcinogenic Metals

A large number of metal ions and their complexes are now considered to be primary carcinogens. The evidence for carcinogenicity of metal ions has been reviewed recently (*1-6*), and no attempt will be made in this paper to go into this subject in detail. The purpose of this review is to consider the properties and coordination chemistry of carcinogenic metal ions, the reactions that they probably undergo in physiological systems, and to consider how the speciation of metal ions in the form of their complexes and chelates may be related to carcinogenic effects. It is first necessary, therefore, to define the scope of this paper in terms of the metals to be discussed.

Carcinogenic metals may be classified in three general categories based on the nature and mechanism of carcinogenesis: radioactive metals, chemical carcinogens, and surface oncogens. This paper will be limited to consideration of metals that function as carcinogens through chemical interaction with biological systems. Metals that achieve carcinogenic effects solely as the result of the production of high-energy particles and/or electromagnetic radiation will not be discussed. Also beyond the scope of this paper are the carcinogenic effects of solid materials, which seem to be more closely related to physical properties and surface characteristics rather than to the chemical nature of the solids.

The periodic distribution of metals that have been recognized as chemical carcinogens is presented in Figure 1. This distribution is interesting in that the metals involved fall into several groups. The more basic metal ions that generally form labile complexes are for the most part not carcinogenic. On the other hand, a large fraction of the fourth period elements excepting groups 1A, 2A, and 7B, (but including many first row transition metals) have been found to have carcinogenic effects. The lanthanides and actinides, that form relatively basic metal ions of +3 and +4 charge, also seem to be generally noncarcinogenic. Details of the nature of the evidence for carcinogenicity of the metals circled in Figure 1 have been reviewed recently (*5, 6*) and the considerations involved will not be repeated here.

The metals that are recognized as the most potent carcinogens are limited to a relatively small number: beryllium, cadmium, nickel, and chromium. Beryllium is the only exception to the generalization mentioned above that the more basic metal ions are not carcinogenic. Evidence for beryllium

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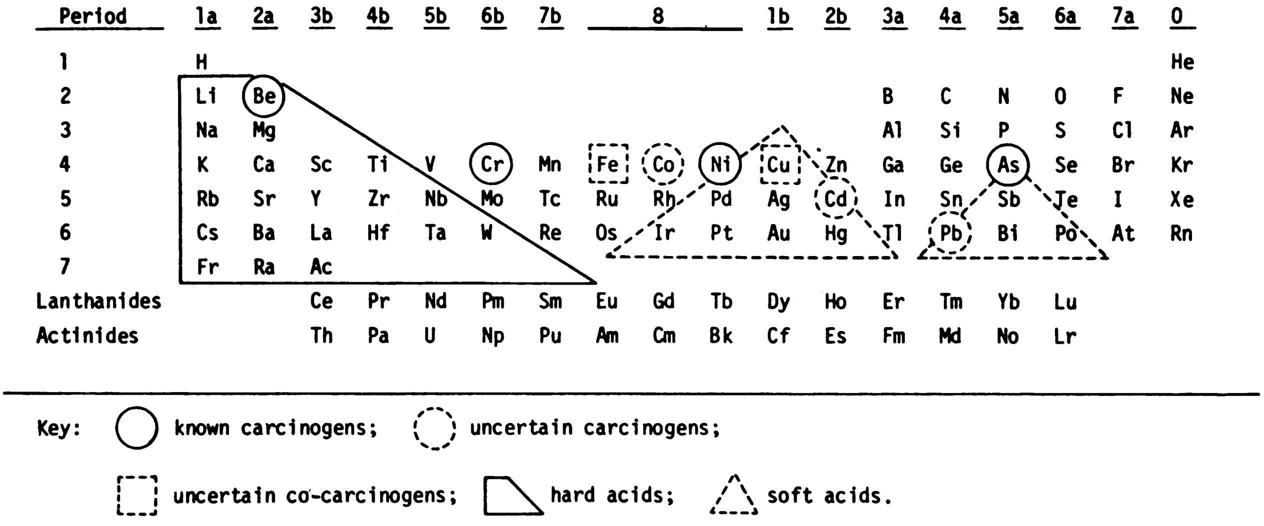

Period 1a 2a 3b 4b 5b 6b 7b 8 1b 2b 3a 4a 5a 6a 7a 0
1 H He
2 Li Be B C N O F Ne
3 Na Mg Al Si P S Cl Ar
4 K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn Ga Ge As Se Br Kr
5 Rb Sr Y Zr Nb Mo Tc Ru Rh Pd Ag Cd In Sn Sb Te I Xe
6 Cs Ba La Hf Ta W Re Os Ir Pt Au Hg Tl Pb Bi Po At Rn
7 Fr Ra Ac
Lanthanides Ce Pr Nd Pm Sm Eu Gd Tb Dy Ho Er Tm Yb Lu
Actinides Th Pa U Np Pu Am Cm Bk Cf Es Fm Md No Lr
Key: known carcinogens; uncertain carcinogens;
uncertain co-carcinogens; hard acids; soft acids.

FIGURE 1. Periodic classification of elements forming compounds having definite carcinogenic effects in man or suggestive of carcinogenicity.

carcinogenicity is well established (*5*, *7*, *8*). Because of its small ionic radius, it would be expected to displace magnesium(II) from enzymes such as RNA polymerase and deoxythimidine kinase. Accordingly, it is not surprising that the carcinogenic effects of beryllium seem to be associated with the high affinity of beryllium compounds for the cell nucleus.

It should be pointed out that the philosophy behind the periodic classification of carcinogenic metals that has been generally employed in the past seems to have involved an inherent assumption that a positive carcinogenic effect of any compound or complex of a metal is sufficient to label the metal as carcinogenic. It is becoming obvious, however, that the interaction of a metal ion with a biological system depends on the nature of the compounds or complexes that are formed, and that changes in the complexing or chelating agent may greatly change its properties, including its carcinogenic effects. A widely recognized example of this principle is the case of chromium, indicated in Figure 1 as a carcinogen. The most frequently observed route involves lung cancer resulting from the inhalation of chromium(VI) by workers in chromium metallurgy and dichromate manufacture. Long induction periods are frequently observed and the actual carcinogenic chromium compound or compounds have not been identified. The nature of the chromium exposure leading to cancer is still controversial.

The conflicting results obtained with various types of metal compounds, and the dependence of the carcinogenic effects observed for a particular metal on the nature of the metal compound involved indicates that clinical results on metal carcinogenicity should be related to the particular metal compound or complex, rather than to the metal in general. Thus it appears that a simple periodic classification illustrated by Figure 1 can be very misleading, and that many of the compounds of the metals indicated as carcinogenic may be quite harmless.

The importance of metal speciation in carcinogenicity is inferred in a recent correlation with metal electronegativities (*9*). It appears that the positively demonstrated and suspected carcinogenic metals are grouped within the electronegativity range 1.1-1.9, with very few exceptions. The ions of the more electropositive metals form very labile complexes and generally have low ligand affinity. The metal ions in the higher electronegativity range form highly covalent bonds with soft donor groups (e.g., mercaptides) and undergo very sluggish exchange reactions with ligands generally found in biological systems. Metal ions in the intermediate electronegativity range have considerable affinity for the nitrogen and oxygen donor groups in many biomolecules, and interact with them with measurable reaction rates. Thus it seems that complex and chelate formation is a common characteristic of metal ions that are found to have carcinogenic properties.

Finally, it should be pointed out that a large number of the carcinogenic metals indicated in Figure 1 are essential to life in trace or moderate concentrations. Metal ions that are essential for certain physiological functions and for the activation of certain enzymes, include nearly all of the first transition series (V, Cr, Mn, Fe, Co, Ni, Cu) as well as Mo, Zn, Cd, and Sn. This interesting dichotomy of essentiality and carcinogenicity and its implications with respect to the Mantel-Bryan (*10*) nonthreshold model of carcinogenesis, and the application of the Delaney clause to food additive legislation has recently been pointed out (*11*).

Formation of Metal Complexes and Chelates

The large amount of experimental data now available on the reactions of electron donors (complexing or chelating ligands) with electron acceptors (hydrogen ion, metal ions) has been correlated in a qualitative manner with the nature and properties of the donors and acceptors through the use of empirical classification involving type (a) ionic, and type (b) covalent, chemical bonding (*12*). Type (a) acceptors consist of the more basic metal ions that tend to form complexes having ionic bonds with little covalent character. The term "hard" was later introduced for the ionic type (a) acceptors and donors, which have low polarizability, and the term "soft" was suggested for type (b) acceptors and donors, which generally have relatively high polarizability (*13*). Thus "hard" and "soft" acceptors were designated as "hard" and "soft" acids, while "hard" and "soft" donors were given the term "hard" and "soft" bases. As would be expected when the principal attractive forces are coulombic, the stabilities of the complexes formed from hard acids and bases increase with increasing ionic charge and decreasing ionic radius. Hard metal ions are generally strongly hydrated in aqueous solution, and form their most stable complexes with negative flouride, oxygen, and nitrogen donors, and to a somewhat lesser extent with negative chloride and sulfur donors. The softest metals on the other hand, such as Ag(I), Hg(I), and Hg(II) form complexes with donor atoms having stability order S > O, P > N, and $I^- > Br^- > Cl^- >> F^-$.

On the basis of these criteria, all the soft acids are situated in two roughly triangular areas of the periodic system (Fig. 1), while the hard acids are generally found in a triangular lefthand area of the periodic system, as indicated. The properties of these acceptors will also vary greatly with charge, as indicated above. Also, it is obvious that the softness of a donor atom will increase with an increase of negative charge. The effect of charge on donors is not as important as it is for acceptors, since there are only two well known binegative donor atoms, the oxide and sulfide anions, O^{2-} and S^{2-}. Details of the effect of ionic charge and other properties of metal ions and ligand donor atoms on the stabilities and covalencies of metal complexes have been described and analyzed by Ahrland (*14*). Various numerical parameters directed toward treating the concept of hard and soft acids and bases in a semiquantitative manner have also be discussed (*15*, *16*).

Another semiempirical correlation of the stabilities of complexes with variation in the electropositive (vs. electronegative) character of metal ions (i.e., hard and soft acids) was pointed out some time ago by Martell (*16*). Such a correlation, indicated in Figure 2, shows increasing stability with increasing electronegativity and increasing charge of the metal ion. Increased electronegativity would be expected to increase covalency of the coordinate bonds formed, since it would result in closer matching of the electronegativies of donor and acceptor. The effect of charge may be in part coulombic (in view of the fact that ligand is negative) and in part due to greater polarization of the negative charge of the ligand toward the metal ion. The use of electronegativity as a parameter is similar in principle to a parameter based on electron affinities used by Ahrland (*14*) to explain relative degrees of hardness and softness of metal ions.

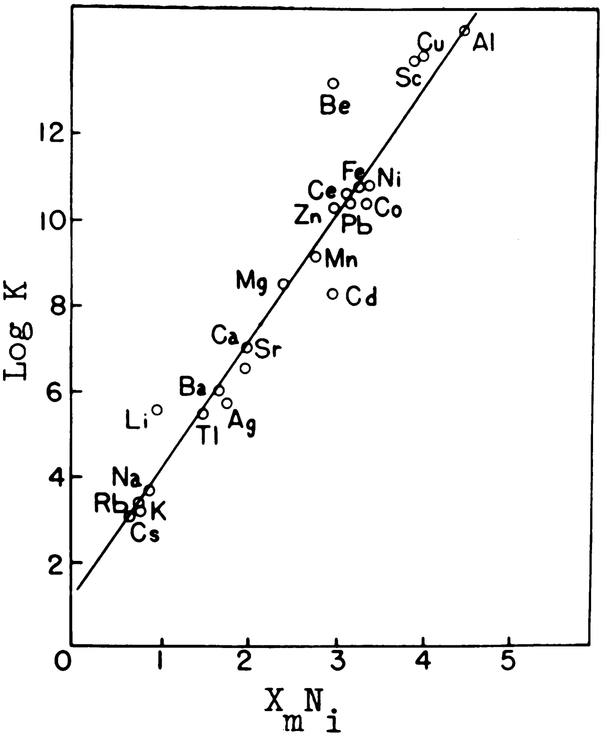


FIGURE 2. Correlation of stability constants, K, of dibenzoylmethane chelates with product of electronegativity of the metal, X_m, and ionic charge, N_i

Metal Chelates and the Chelate Effect

It is widely recognized that metal chelates are much more stable in aqueous solutions than simple complexes of the same metal ions with monodentate ligands containing similar donor atoms. Thus any increment of stability due to the "chelate effect" is superimposed on the factors cited above, such as electronegativity and hard and soft character of donors and acceptors. Since nearly all of the natural and artificially introduced coordination compounds that are found in biological systems are metal chelates, it is appropriate at this point to consider the factors that control stability and other properties of metal chelate compounds.

The coordination tendencies of metal ions in aqueous solution result in the formation of aquo complexes containing several coordinated water molecules, and which are designated as "aquo" metal ions. The majority of the frequently encountered metal ions have coordination numbers of six, and are bound to six water molecules usually arranged in a manner more or less approximating a regular octahedron. Relatively large, highly-charged metal ions, such as those of the actinides and lanthanides, and also Zr(IV), and Hf(IV) have coordination numbers of eight, while other metal ions of lower charge, such as those of Zn(II), Be(II), Al(III), Ga(III), and Mg(II) seem to have coordination numbers of four or six, depending on the ligands and the reaction conditions. For metal ions of intermediate and low basicity the covalent character of the coordinate bonds is sufficient to maintain a fixed number of donor groups in a specific geometric arrangement about the metal center. For the more basic metal ions of intermediate or low ionic charge, the number and geometrical arrangement of coordinated donor groups may vary considerably, and a dynamic equilibrium involving a distribution of structures may be assumed to exist.

A metal chelate compound is merely a metal complex or coordination compound in which two or more of the donor atoms coordinated to the metal ion are bound together by some kind of chemical linkage. According to this general description, metal chelate compounds would not have special properties that distinguished them from simple complexes. On the other hand when a chelate ring meets certain structural and constitutional specifications, its stability is increased and it may also have additional properties that cannot be achieved in simple complexes with independent donor groups. The principal properties of metal chelate compounds are described below.

The formation of highly stable metal chelates in aqueous solution have important applications in biological systems. The use of chelating ligands with a sufficient number of donor groups to match the coordination requirement of a metal ion makes it possible to achieve 1:1 stoichiometry in the formation of a chelate compound—an important property if stability of the chelate compound is to be maintained in extremely dilute solutions. The conceptual and thermodynamic basis for the special properties of metal chelate compounds has been described in a number of reviews (*17-19*).

For a metal ion of coordination number six, for example, the reactions involving monodentate and sexadentate ligands may be compared, as follows:

$$M + 6A \rightleftarrows MA_6 \qquad \beta_{MA_6} = [MA_6]/[M][A]^6 \quad (1)$$

$$M + L \rightleftarrows ML \qquad \beta_{ML} = [ML]/[M][L] \quad (2)$$

For the complex MA_6, the units of β_{MA_6} are molarity to the negative sixth power, and the degree of formation of the complex is proportional to the sixth power of the free ligand concentration, which in very dilute solution can become a vanishingly small number. The degree of formation of the chelate compound ML of the sexadentate ligand, however, is much less sensitive to concentration, and decreases linearly with the first power of the free ligand concentration.

The high stability of metal chelates relative to metal complexes in dilute solution is clearly related to the values of the entropies of dilution of the complexes and chelates relative to the entropies of dilution of the dissociated species with which they are in equilibrium. This experimental fact (that metal chelates are much less dissociated in dilute solution) is illustrated in Table 1, which compares the degrees of dissociation of coordination compounds containing zero, three, and five chelate rings. An average chelate effect of 10^2 per chelate ring is assumed as the basis of the arbitrary stability constants employed—a result that would be achieved if the donor groups of the ligands have approximately equivalent metal ion affinity. The superior stability of the metal chelate in dilute solution, and the striking effect of increasing the number of metal chelate rings, is dramatically illustrated by a comparison of the percent dissociation of the metal chelates and complexes indicated for unit and thousandth molar solutions.

As pointed out by Adamson (*20*), the entropy-related chelate effect, which was assigned a value of 10^2 in log β per chelate ring, is a result of the use of unit molality ($\cong$ unit molarity) as the standard

Table 1. Comparison of dissociation of metal complexes and chelates in dilute solution.

Donor groups per ligand	No. of chelate rings	Equilibrium quotient	β_x	1.0 *M* Complexes Free [M]	1.0 *M* Complexes %Dissociation	1.0×10^{-3} *M* Complexes Free [M]	1.0×10^{-3} *M* Complexes % Dissociation
1	0	$\frac{[MA_6]}{[M]\,[A]^6}$	$10^{18}\ M^{-6}$	6×10^{-4}	6×10^{-2}	2×10^{-4}	20
2	3	$\frac{[MB_3]}{[M]\,[B]^3}$	$10^{24}\ M^{-3}$	5×10^{-7}	5×10^{-5}	1×10^{-7}	1×10^{-2}
6	5	$\frac{[ML]}{[M]\,[L]}$	$10^{28}\ M^{-1}$	1×10^{-14}	1×10^{-12}	3×10^{-16}	3×10^{-11}

reference state for solutes in aqueous solutions. The value of the chelate effect would vary considerably if some other concentration were selected as the reference state. It would increase considerably for 10^{-3} M and would vanish for unit mole fraction standard state. Regardless of these considerations, the relative degrees of dissociation of the model compounds in Table 1 remain as an experimental fact.

Other Factors Influencing Stabilities of Metal Complexes and Chelates

Table 2 indicates that there are many factors in addition to the entropy-based chelate effect that must be taken into account in order to fully understand metal-ion affinites of multidentate ligands. Mutual coulombic repulsions between donor groups in metal chelates are important, and the extent to which these repulsions are overcome in the free chelating ligand relative to the coulombic repulsions that the corresponding unidentate ligands must undergo in complex formation is a manifestation of the enthalpy-based chelate effect. This property, which greatly increases stability constants of chelates, is inherent in the enthalpies of formation of the chelating agents in solution. This enthalpy effect is developed to the highest possible degree in macrocyclic and cryptand ligands in which the donor groups are held at geometric positions that are relatively close to the positions that they would have when combined with metal ions. Thus stability and specificity of both natural and synthetic multidentate ligands are achieved by the arrangement of donor groups in positions favorable for satisfying the coordination requirements of the appropriate metal ions. In biological macromolecules this objective may be achieved by the positioning of donor groups in favorable geometric arrangements through, for example, the folding of a polypeptide chain. Specificity of synthetic ligands depends on the development of a molecular framework that will achieve similar results, either through ring formation or the utilization of rigid aromatic structures.

Donor Groups

Examples of donor groups that may be built into synthetic and natural ligands are shown in Figure 3. These constitute a partial list involving only the more common donor groups. For the donors involving oxygen atoms, for example, analogous ligands in which sulfur atoms replace one or more oxygens are also possible, and many such ligands are avail-

Table 2. Factors influencing solution stabilities of complexes and chelates.

Enthalpy effects	Entropy effects
Variation of bond strength with electronegativities of metal ions and donor atoms of ligands	Number of chelate rings
Ligand field effects	Size of the chelate ring
Enthalpy effects related to the conformation of the uncoordinated ligand	Arrangement of chelate rings
Steric and electrostatic repulsions between ligand donor groups	Changes of solvation on complex formation
Heats of desolvation of metal ion and ligand	Steric interferences with chelates ring formation
Other coulombic forces involved in chelate ring formation	Entropy variations in uncoordinated ligands
	Effects resulting from differences in configurational entropies of the free and coordinated ligands

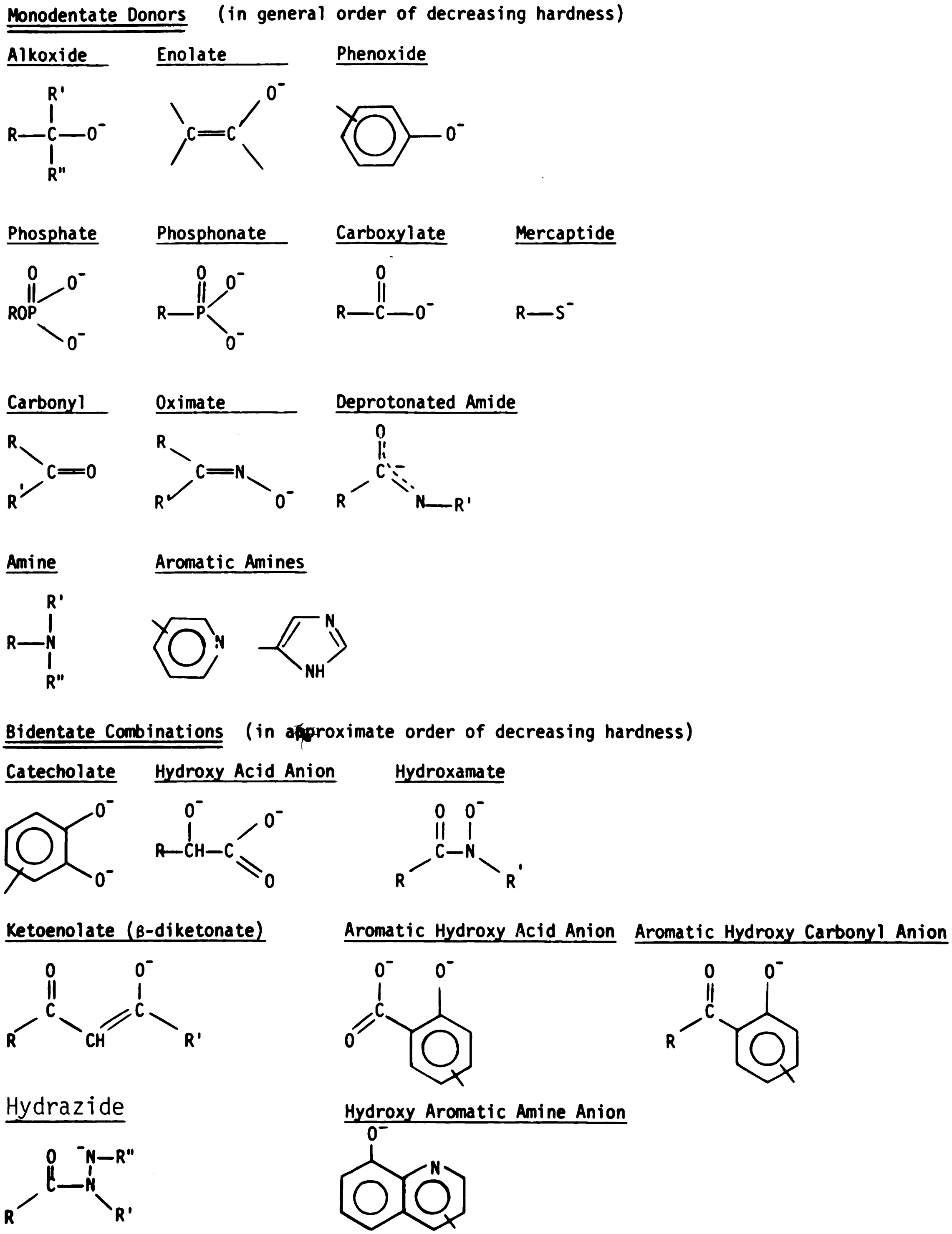

Monodentate Donors (in general order of decreasing hardness)
Alkoxide
Enolate
Phenoxide
Phosphate
Phosphonate
Carboxylate
Mercaptide
Carbonyl
Oximate
Deprotonated Amide
Amine
Aromatic Amines
Bidentate Combinations (in approximate order of decreasing hardness)
Catecholate
Hydroxy Acid Anion
Hydroxamate
Ketoenolate (β-diketonate)
Aromatic Hydroxy Acid Anion
Aromatic Hydroxy Carbonyl Anion
Hydrazide
Hydroxy Aromatic Amine Anion

FIGURE 3. Types of donor groups in natural and synthetic ligands.

able. The bidentate donor groups are those in which the donor atoms function in a cooperative fashion through resonance interactions. Thus for bidentate functional groups containing an unsymmetrical formal charge, the resonance effects distribute the charge partially or equally between the donor atoms, depending on the molecular structure, thus making both donor atoms effective in metal ion coordination. Several of these bidentate donor groups are synthesized in microbial systems for metal ion transport. Examples are the microbial iron(III) carriers that contain one or more catechol or hydroxamic acid groups (*21-26*). These functional groups have also been incorporated into synthetic analogs of the microbial iron carriers for the treatment of iron overload disease (*27*, *28*).

Examples of Chelating Ligands

The polyaminopolycarboxylates indicated in Table 3 constitute a familiar series of synthetic ligands that have been widely used in biological systems as well as for analytical and commercial purposes. The first three members of the series, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and diethylenetriaminepentaacetic acid (DTPA) are well known. The two higher members of the series are available only in small quantities for experimental purposes.

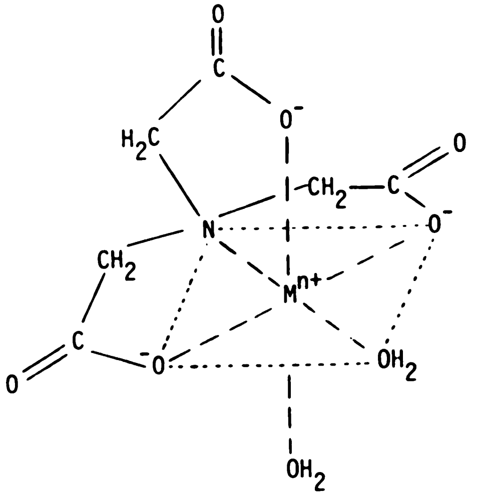
For metal chelates in which all of the amino and carboxylate donor groups of the ligands in Table 3 are coordinated to the metal ion, there are n-1 five-membered chelate rings, where n is the total number of nitrogen and oxygen donors (one oxygen per carboxylate group). This extent of chelate ring formation is generally achieved for most of the well-known di- and trivalent metal ions, provided that the coordination number of the metal ion does not exceed n. Typical examples of metal chelates of NTA, EDTA and DTPA are illustrated by I-IV.

The data in Table 3 indicate that continued increase in the number of donor atoms in ligands of this type does not produce a parallel increase in the stability constants of chelates formed with divalent metal ions. A decrease eventually occurs, with the maximum value for Ca(II) ion obtained with DTPA. For Cu(II) the maximum stability occurs with triethylenetetraminehexaacetic acid (TTHA). For metals of higher ionic charge, the maximum stabilities are not known because of the lack of stability constant data for the higher ligands, but probably occur with tetraethylenepentamineheptaacetic acid (TPHA) or the next higher member of the series. This type of behavior is rationalized by the view that for basic metal ions such as Ca^{2+}, La^{3+}, and Th^{4+}, the stabilities of the corresponding chelates of these ligands depends on the ability of the ligands to form an "ionic cage" about the metal ion. For moderate to low coordination number, this objective is best achieved with EDTA or DTPA. For TTHA some of the carboxylate donor groups will not be coordinated, probably leaving an unbound pair at one end of the ligand. On that basis TTHA would present only four negative charges to the calcium(II) ion while DTPA would provide five. Similar considerations would be expected for the higher members of this series of ligands in the coordination of basic metal ions of higher charge and coordination number, such as the tripositive lanthanides and tetrapositive actinides.

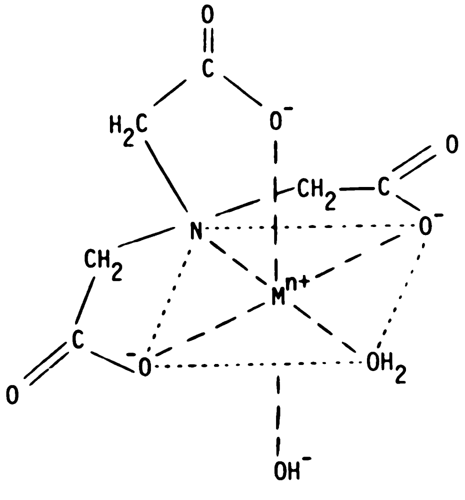
It is apparent from the above remarks that the ligands in Table 3 form stable chelates with a wide variety of metal ions but are not very specific: i.e., they do not effectively distinguish between various metal ions. For metal ions of increasingly higher charge (increasing "hard acid" character), higher stability can be obtained by simply increasing the number of carboxylic acids, that are only moderately hard basic groups. In the design of new chelating ligands, higher selectivity may be achieved by replacing one or more carboxylate groups by one or more coordinating groups having more selective coordinating properties. Greater stability and selectivity have been achieved by the introduction of functional groups having high affinity for the metal ion under consideration. An example of how ligands may be varied in this manner follows.

It has been known for a long time that phenolate oxygen donors have very high affinity for the ferric ion. Highly specific sexadentate ligands for the Fe(III) ions have been synthesized by modifying the EDTA structure so as to provide two carboxylate and two phenolate donor groups attached to the basic ethylenediamine framework. The ligands ethylene-*N*,*N'*-bis-2-(*o*-hydroxphenyl)glycine (EHPG) (*29*) and *N*,*N'*-bis(*o*-hydroxybenzyl)ethylenediamine-*N*,*N'*-diacetic acid (HBED) (*30*), illustrated in Table 4, have affinities for Fe(III) from 9 to 14 orders of magnitude over those of EDTA. On the other hand the phenolate ligand has little selectivity over EDTA for other metal ions such as those of Zn(II) and Ni(II). In the case of Ca(II), the phenolate analogs are poorer ligands than EDTA. The much higher effectiveness of *N*,*N'*-(bis) (2-hydroxybenzyl)-ethylenediamine-*N*,*N'*-diacetic acid (HBED) over EHPG for iron(III) is considered to be due to the much more favorable steric orientation in HBED of the carboxylate groups, which bind the ferric ion much more strongly than is possible with EHPG.

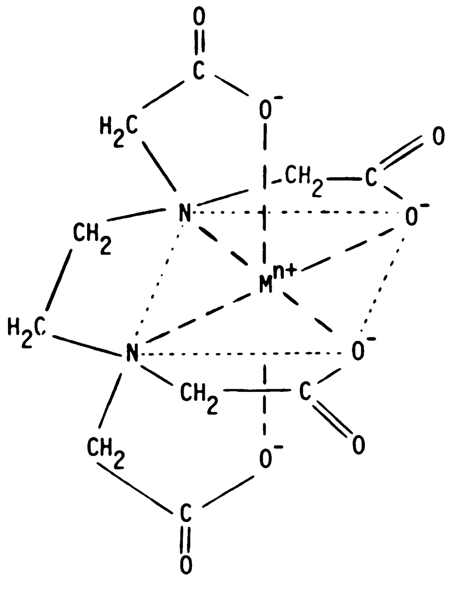
The high affinity of HBED and EHPG for iron(III) is due to the presence of two phenoxide groups in



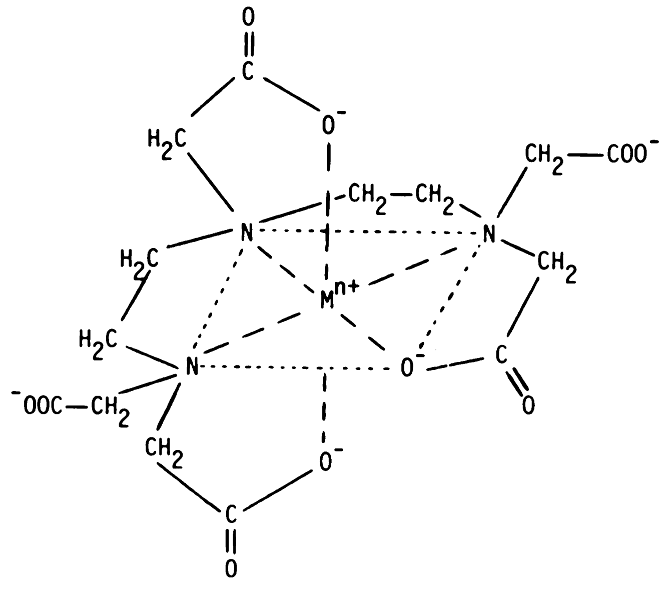
I NTA Chelate



II Hydroxo NTA Chelate



III EDTA Chelate



IV DTPA Chelate

each ligand. These donor groups are "hard bases" and match the "hard acid" character of the iron(III) ion. It was pointed out above that oxygen atoms of catechols and hydroxamic acids are also hard bases and are highly effective for iron(III). Two examples of microbial ligands containing hydroxamate (*24*) and catecholate (*25*) donor groups are illustrated by formulas V and VI, respectively. The six oxygen donors in each ligand are arranged octahedrally around the iron(III) ion. The higher stability of the catecholate-type chelate [enterobactin-iron(III)] is due to the higher basicity and higher ionic charge of the six phenolate donor groups in dissociated enterobactin. The relative stabilities in biological systems, however, are not as different as is indicated by the relative values of the stability con-

Table 3. Stabilites of 1:1 chelates of NTA and EDTA and Their analogs (25°C, 0.10*M* ionic strength).

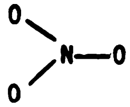
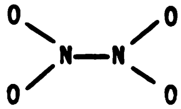
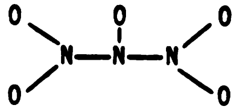
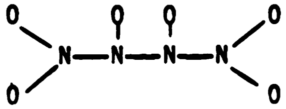
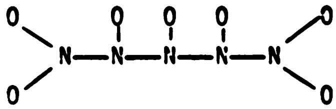
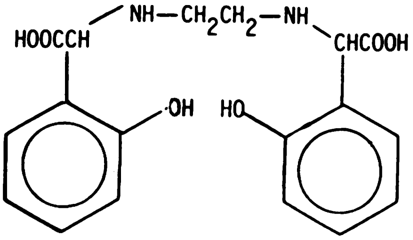
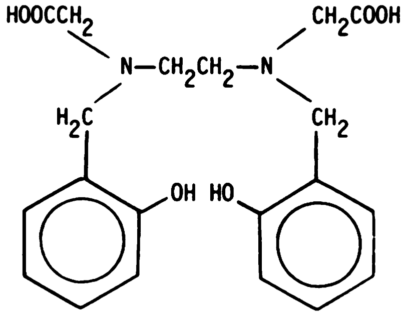
Ligand		Log formation constants			
		Ca^{2+}	Cu^{2+}	La^{3+}	Th^{4+}
NTA		6.57	12.96	10.47	12.4
EDTA		10.4	18.7	15.2	23.2
DTPA		10.7	21.1	19.5	28.78
TTHA		9.9	20.3	23.1	31.9
TPHA		9.0	~20	>27	>>32

Table 4. Stabilities of chelates of EDTA analogs containing phenolic groups (25°C; 0.10*M* ionic strength)

Ethylenebis-*o*-hydroxyphenylglycine
(EHPG)

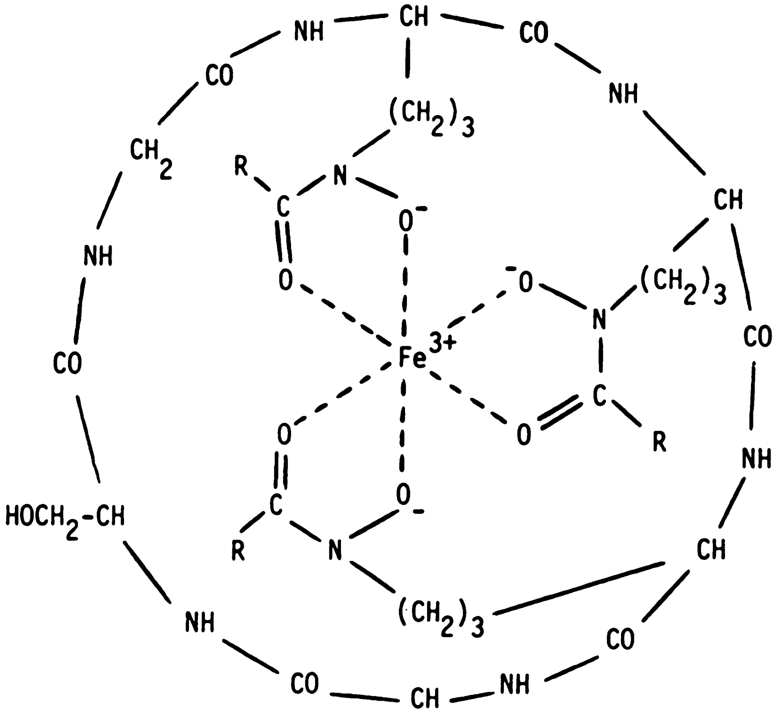


N,N′-bis (*o*-hydroxybenzyl)ethylenediamine-N,N′-diacetic acid (HBED)

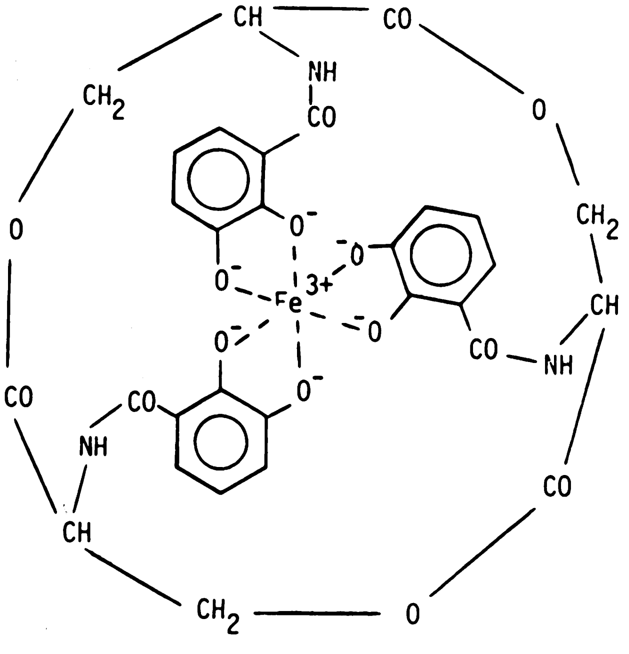


	$\log K_{ML}$	$\Delta \log K^a$	$\log K_{ML}$	$\Delta \log K^a$
Cu^{2+}	23.90	5.20	21.38	2.68
Ni^{2+}	19.60	1.14	19.31	0.79
Zn^{2+}	16.80	0.36	18.38	1.93
Ca^{2+}	7.20	-3.41	9.29	-1.32
Fe^{3+}	33.91	8.91	39.68	14.68

[a] $\Delta \log K = \log K_{ML} - \log K_{ML'}^{EDTA}$



V Ferrichrome A [R = $CH=C(CH_3)CH_2COOH$]



VI Iron (III)-enterobactin Chelate

stants given. This moderating effect which is due to proton competition for the ligand donor groups, is explained below.

Selectivity through Design of Molecular Framework of Coordinating Ligand

The chelating ligands described above are generally flexible molecules that have quite different structures in solution in the absence and in the presence of metal ions. Thus the process of metal chelate formation requires reorientation of the ligand structure and close approach of polar and charged donor atoms. These requirements result in adverse enthalpy (coulombic repulsion) and entropy effects (loss of rotational and vibrational freedom). Both affinity and specificity for metal ions may be greatly increased by the use of more rigid molecular frameworks so that the donor groups are positioned very close to the orientations that they would have in the metal chelate. Well-known examples of this type of ligand are provided by the porphyrins and the phthalocyanines, which coordinate metal ions with four completely resonance-linked aromatic nitrogen donor atoms arranged in a square plane around the central metal ion. In many cases the coordination requirements of the coordinated metals are satisfied by two additional monodentate ligands in the axial positions. The literature on the coordination chemistry of these complexes is very extensive and the chemistry of these systems will not be described in this paper. Interested readers are referred to a recent review (*31*) and references cited therein.

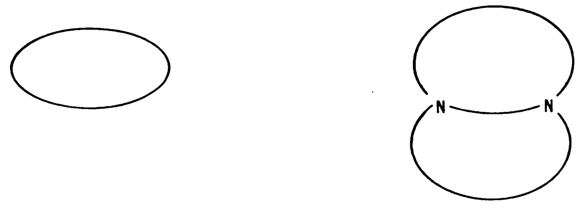
The "crown ethers", other macrocyclic ligands, and the cryptate ligands are examples of cyclic chelating agents that are more flexible than the porhpyrins and phthalocyanines. However, the ring systems in these ligands may be varied so as to produce cavities surrounded by donor atoms in sufficiently restricted positions to discriminate between metal ions differing in ionic radius. The following is a brief discussion of these ligands and their chelates because of their close relationship to the ionophores.

The so-called crown ethers are macrocyclic ligands containing ether oxygens as coordinating donor groups surrounding more or less well-defined cavities. The special property of these ligands is their ability to form stable coordination complexes of alkali metal and alkaline earth ions. The crown ethers, and other macrocyclic ligands containing other donor atoms such as nitrogen and sulfur, consist of single rings with varying numbers of coordinating donor atoms. While these ligands may form two-dimensional rings, the aliphatic bridges between the donor atoms are flexible and three-dimensional complexes are readily formed. Ligands containing three polyether (or polyamine or polythioether) strands joined by two bridgehead nitrogens provide three-dimensional cavities that are more well-defined than is the case for the simple macrocycles, and have higher specificities for metal ions of varying ionic radius. The inclusion complexes formed are called cryptates, and the metal-free ligands are designated as cryptands.

Representative topologies of crown and cryptand ligands are given in Figure 4. The simple macrocycle A may have a wide variety of coordinating heteroatoms. For the polyethers, rings varying from 9 to 60 atoms, with from 3 to 20 oxygen atoms have been synthesized. With two bridgehead nitrogens cryptands represented by B may be synthesized so as to provide one or more coordinating atoms in each bridge. With four bridgehead nitrogens, the cryptand molecule may have a cylindrical or spherical shape, as indicated by C and D, respectively. It is seen that these structures can be expanded with additional bridges and bridgehead atoms to form a wide variety of conformations. With variations in the number of kind of donor atoms in each bridge, it is seen that the possible number of cryptand ligands is almost limitless.

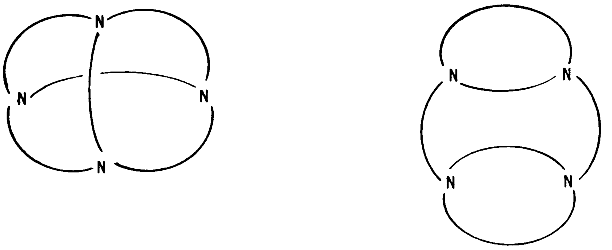
The Macrocyclic Effect

The stability constants for the formation of the Cu(II) and Ni(II) chelates of typical four-nitrogen macrocycles and their open-chain tetramine analogs are presented in Table 5. The remarkably higher stability of 5-7 orders of magnitude for the macrocyclic complex is typical for comparisons of this nature. The term "macrocyclic effect", which may be up to ten times larger than the chelate effect for analogous mono- and polyamine complexes, was ascribed by Margerum et al. (*32*, *33*) to differences in the degrees of hydration of the open-chain and cyclic ligands, the latter being much less solvated. Thus formation of the macrocyclic complex takes place with much less expenditure of desolvation energy. These arguments are based on measured thermodynamic parameters of formation of chelates of the type illustrated in Table 5, and the assumption that the thermodynamic properties of the final metal chelates are very much alike, with or without the additional chelate ring. This effect may account for a major part of the macrocyclic effect. It is suggested here, however, that entropy considerations are also important factors contributing to higher stability of the macrocyclic chelate. The



A: Macrocycle

B: Simple cryptand



C: Spherical cryptand

D: Cylindrical cryptand

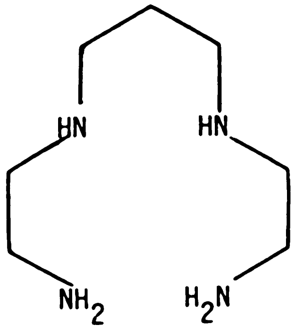
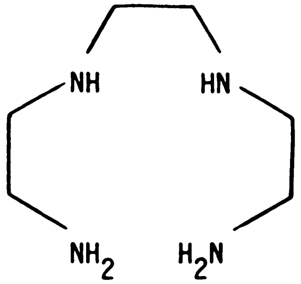
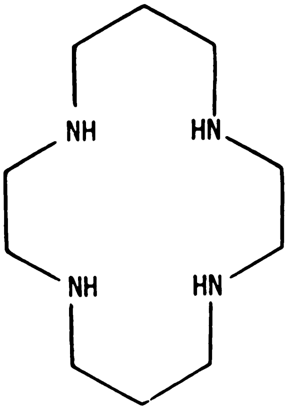
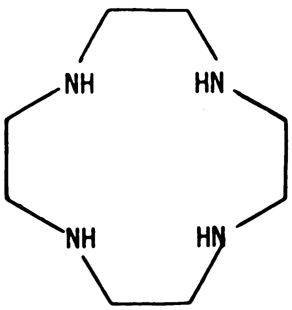
FIGURE 4. Representative topologies of crown and cryptand ligands.

open chain ligand has an extended configuration, which is not indicated in Table 5, and formation of the chelate results in significant negative entropy effects because of the restriction of rotational and vibrational motion of the ligand. Such restrictions do not occur in the formation of the macrocyclic chelates, since the free ligands exist initially in conformations which are close to those that are required for coordination of the metal ion.

The Cryptate Effect

The "cryptate effect" has been described by Lehn and co-workers (*34*, *35*) as the increase in the stability of a macrocyclic chelate resulting from the formation of an additional connecting bridge to form macrobicyclic ligands, or cryptands. This effect, which is illustrated in Table 6, is even larger than the macrocyclic effect for the same metal ions (Table 7). The factors described above that produce the macrocyclic effect may be further extrapolated to explain the even higher stabilities of cryptates. This increase is at a maximum when the three-dimensional cavity between the donor atoms closely fits the dimensions of the metal ion under consideration. From a thermodynamic point of view the cryptate effect is due to both enthalpic and entropic factors favoring formation of the metal cryptate. The lower hydration of the cryptand (relative to open chain and macrocyclic ligands) is an important factor. It has been pointed out by Lamb et al. (*36*), however, that other enthalpic factors must be involved since the cryptate effect has been shown to increase with a decrease of dielectric constant. It is now suggested that this "other enthalpy factor" is the overcoming of the coulombic repulsions between the polar or charged donor groups in the cryptand relative to macrocyclic and open chain ligands, in which the polar groups are free to move farther apart to a much greater extent than is possible for the analogous metal cryptates. It is also suggested that there is an inverse relationship between the hydration energy and coulombic repulsion enthalpic terms. In solvents of high dielectric

Table 5. Formation constants of macrocyclic chelates and of analogous chelates of open-chain polyamines.

Ligand L	Log K (NiL)	Ligand, L	Log K (CuL)
	15.3		20.1
	22.2		24.8

constant the polar groups will be highly solvated and the coulombic repulsions between the donor groups will be mitigated by the solvent molecules associated with the ligand donor atoms. It is these solvent molecules that must be displaced in the formation of a metal complex or chelate. On the other hand, in solvents of low dielectric constant there is less solvation, so that this effect must be less important. Because of the lower solvation however, the coulombic repulsions between the negatively charged donor groups that must be overcome in formation of a metal complex or chelate are relatively much larger. Both of these enthalpic factors work against metal ion coordination, and both are at a minimum in the formation of metal cryptates.

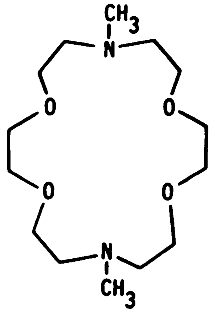
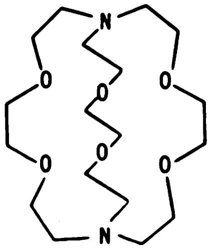
Entropy factors also provide important contributions toward the higher stabilities of cryptates, since the formation of a cage of donor atoms prior to coordination of the metal ion results in less loss of vibrational and rotational entropy in the process of metal ion coordination. Further rigidity may be built into the framework of both macrocyclic ligands and cryptands by incorporating aromatic rings or other groups that restrict rotation of various parts of the ligand molecule, thus further decreasing the entropy loss on coordination, and favoring higher stabilities of the macrocyclic and cryptand chelates.

While cryptates represent the extreme in the achievement of molecular structures that favor metal complex formation, there are kinetic disadvantages to their application in chemical and biological systems. The closer the fit of the wrap-around ligand to the metal ion, the slower will be the rate of formation and dissociation of its chelates, and the slower will be the rate at which one metal ion displaces another from such complexes. This problem may not be a very serious one for many of the labile, highly ionic chelates formed by the alkali and alkaline earth metal ions. For transition metal ions having an appreciable covalent component in their coordination compounds, the protective ligand shell around cryptate and even some macrocyclic chelates may render the metal ion quite inert to dissociation and exchange reactions.

Ionophores

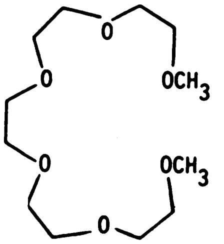
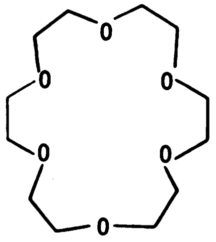
Ionophores are natural and synthetic ligands that can transport metal ions across low-dielectric constant barriers such as lipids, organic solvents, and biological membranes. This functional definition encompasses a wide variety of possible chemical

Table 6. Examples of the cryptate effect.[a]

Ligand	log *K*				
	Na^+	K^+	Ca^{2+}	Sr^{2+}	Ba^{2+}
	3.26	4.38	4.4	6.1	6.7
	7.21	9.75	7.60	11.5	12.0

[a] 95 vol % methanol.

Table 7. Macrocyclic effect for analogous metal Ions as in Table 6.[a]

	log *K*	
	K^+	Ba^{2+}
	1.96	2.34
	5.35	6.56

[a] 90 vol % methanol.

structures. The best known ionophores at present typically have ether or carbonyl oxygens arranged in the form of a cage or ring around a cavity sufficiently large to hold and coordinate various metal ions. In addition to the neutral but polar ether and carbonyl oxygens, certain ionophores contain one (or more) carboxylate groups, that assist in charge neutralization as well as coordination of the metal ion. Others may have aliphatic hydroxyls that seem to assist with maintaining the desired conformation through hydrogen bonding, but coordination of the metal ion by hydroxyl oxygen is not precluded. The ionophores studied thus far are either macrocyclic ligands or open chain compounds that can assume a macrocyclic conformation through head-to-tail hydrogen bonding. Although synthetic cryptates have not generally been considered as ionophores, there is no reason why they should not function as well or better than the macrocyclic ionophores, although for certain metal ions there may be kinetic problems, as mentioned above. Work on the use of cryptand ligands for metal ion transport in low-polarity media is no doubt in progress at the present time in many laboratories.

The properties of ionophores and their metal chelates have been described in detail in several recent reviews (*38-41*). For the purposes of this paper only a few typical examples will be discussed: valinomycin (VII), a macrolide actin (VIII), dicyclohexyl-18-crown-6 (IX), and monensin (X).

Valinomycin is a cyclic dodecadepsipeptide consisting of alternating amino acid and hydroxy acid residues condensed through the carboxylic acid, amino, and hydroxyl groups. The peptide carbonyls form a three-dimensional cage that accommodates K^+ more efficiently than Na^+, with a displacement constant $K_d = [Na^+][KCr^+]/[K^+][NaCr^+]$ of about 10^4.

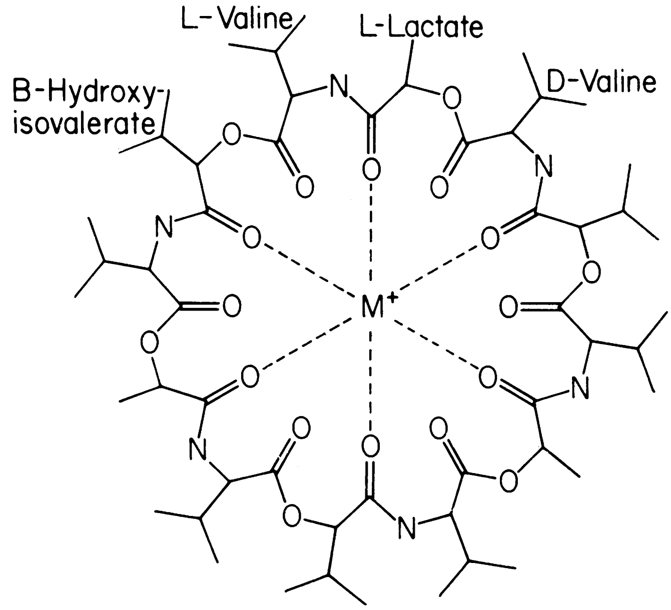
The macrolide actins (VIII) are cyclic tetraesters that provide eight oxygens arranged approximately at the apices of a cube for coordination of a central metal ion. The coordinating oxygens are derived from alternating ester carbonyls and heterocyclic ether oxygens. The synthetic crown polyethers, represented by dicyclohexyl-18-crown-6 (IX), are somewhat less efficient as chromophores than some of the natural compounds, but have the advantage of higher chemical stability since they are not subject to hydrolytic attack. It seem likely that the efficiency of the crown ethers may be improved by the introduction of alkyl groups and the careful building of suitable chirality into the parent polyethers.

Monensin (X) is an example of the naturally occurring carboxylic acid ionophores. It consists of a linear arrangement of ether-containing heterocyclic rings. The chirality of these ring systems and adjacent asymmetric carbons favors a cyclic arrangement of the molecule, which is further stabilized by head-to-tail hydrogen bonding. There are also two hydroxyl oxygens that seem to be involved in metal coordination.

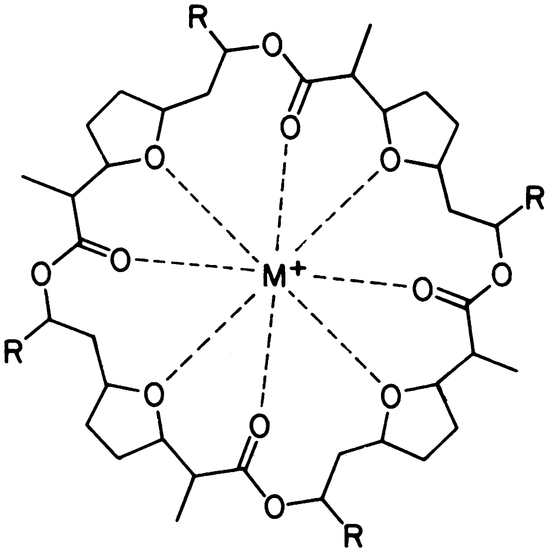
Because of the membrane permeability of their metal complexes, ionophores have unique biological activity in altering transmembrane metal ion gradients and electrical potentals. Various ionophores differ in ion selectivity as the result of different geometries of the donor atoms surrounding the metal ion-accepting cavity. The metal ion complexing and transport functions of ionophores has led to an increasing list of applications as novel drugs for many purposes, including cardiovascular applications. They are also of interest from a purely chemical and bioinorganic point of view for studies of metal chelation processes *in vivo* and *in vitro*, as well as in chemical and biological media of varying ionic strength. In addition to providing new tools for biological research, ionophors now have practical applications as additives to poultry and livestock feed for increasing the efficiency of meat production, and for the development of potentially useful drugs in man. In view of the rapid large-scale increases in commercial ionophore usage, it seems urgent that our knowledge of their chemical and biochemical reactions be increased so as to improve our understanding of the nature of their pharmacological and toxicological effects.

Effective Stabilities of Metal Chelates in Solution

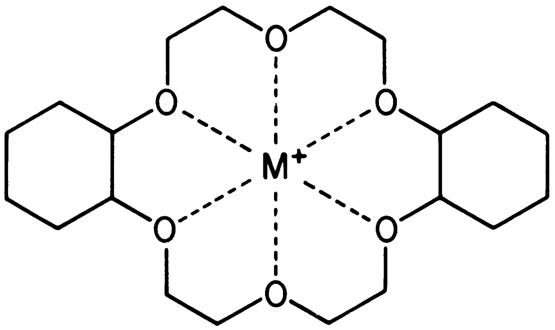
As indicated above, the formation of metal complexes and chelates in biological systems is controlled by the stability (equilibrium) constants (K_n) for combination of metal ions with each ligand present. It is a common fallacy to consider that the ligands with the highest stability constants for a given metal ion will result in the highest degree of formation of the corresponding metal complexes. There are many competing reactions that prevent the existence of such a simple parallel relationship between published stability constants and degree of formation of the chelate compounds. Stability constants are generally directly applicable only under special conditions which are most favorable for chelate formation—in the absence of competing ions and in the optimum pH region in which the ligand is fully dissociated. Generally, the effectiveness of a chelating agent *in vivo* is reduced substantially by competing ions that may be present in the



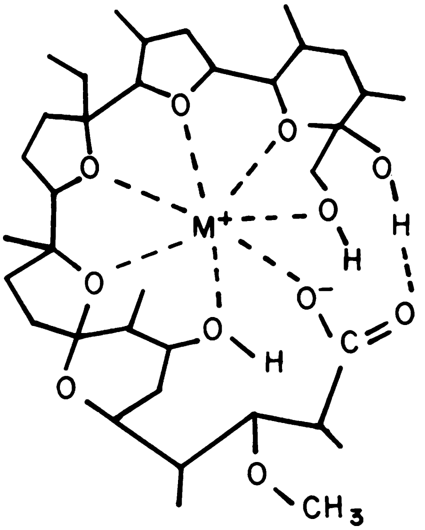
VII Valinomycin



VIII Macrolide Actins



IX Metal Complex of Dicyclohexyl-18-crown 6



X Monensin

biological system under consideration. Neglecting other competing ligands, calcium ions, about $10^{-3}M$ in physiological systems, hydrogen ions ($10^{-7}M$) and hydroxide ions ($10^{-7}M$) constitute the most serious interferences which compete with either the ligand for the metal ion or with the metal ions for the ligand. Thus the greater the basicity of the chelating agent, the greater is its affinity for the metal ion, but this effect is paralleled by a higher affinity for protons, so that hydrogen ions may provide very strong competition for the metal ion, except for relatively high pH conditions under which the ligand is fully dissociated (deprotonated). This competition is due to the formation of acid forms of the ligand, thus lowering the concentration of the free basic form of the ligand available for metal binding. It should also be noted that these competing reactions may vary considerably from ligand to ligand. The relative effectiveness of various ligands in coordinating metal ions will differ considerably from the relative stability constants if they exhibit considerable differences in proton affinity.

In the design of ligands to achieve selectivity of metal binding in biological systems, it is helpful to calculate interferences by the use of the appropriate ligand protonation constants, the binding constants of competing metal ions for the ligand(s) in question and the hydrolysis constants of the metal ions.

In the case of proton interference, the term α_L, which represents the fraction of ligand in its completely deprotonated form, is frequently employed

$$[L^{m-}] = T_L \alpha_L \qquad (3)$$

where T_L is the sum of the concentrations of free ligand and its protonated species (i.e., the sum of the concentrations of all non metal-coordinated forms of the ligand). The formation of the protonated species is governed by the corresponding equilibrium constants.

$$\beta_i^H = [H_iL]/[H^+]^i[L] \qquad (4)$$

$$\alpha_L = (1 + [H^+]\beta_1^H + [H^+]^2\beta_2^H + \ldots . [H^+]^n\beta_n^H)^{-1} \qquad (5)$$

The corresponding expressions for calcium ion and hydroxide ion interference are given by Eqs. (6) and (7), respectively, and the effective binding constant, K_{eff}, of the metal chelate is given by Eq. (8).

$$\alpha_{CaL} = ([Ca]\beta_{CaL})^{-1} \qquad (6)$$

$$\alpha_M = (1 + [OH^-]\beta^{OH}_1 + [OH^-]^2\, \beta^{OH}_2 + \ldots [OH^-]^m\beta^{OH}_m)^{-1} \qquad (7)$$

$$\log K_{eff} = \log \beta_{ML} - n \log(\alpha^{-1}_{CaL} + \alpha^{-1}_{L}) - \log \alpha^{-1}_{M} \qquad (8)$$

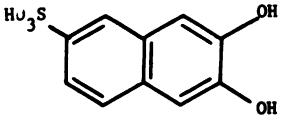
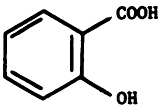
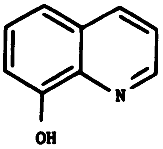
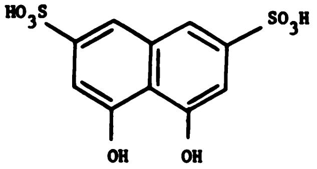
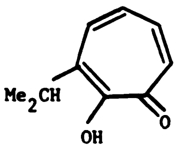
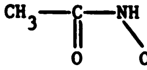
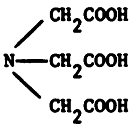
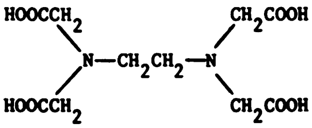
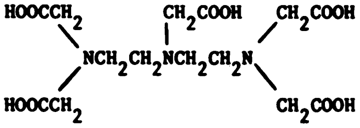
Equation (8) takes into consideration only the soluble mononuclear hydroxy complexes of the metal. In concentrated solutions, and in the presence of a precipitate of the insoluble hydroxide of the metal ion, the situation is considerably more complex. Generally, however, the conditions are such that only soluble mononuclear hydroxy metal species need be considered, and interference by hydroxide ion, as well as hydrogen ion, is dependent only on pH. If one uses 7.4 as the pH value of greatest interest for physiological systems, the effective metal binding constants of natural and synthetic ligands may be compared. Some representative values are given in Table 8, in which Fe^{3+} is selected as an important biological metal ion which as a hard acid requires hard bases for effective binding in aqueous systems. The ligands containing phenolic groups (1-5 and 11-13) have very high proton affinities and the value of K_{eff} is considerably lower than the stability constants β_n. For the aminopolycarboxylic acid ligands (7-10) there is less proton competition than for the phenols, but the K_{eff} values are reduced by strong Ca^{2+} competition.

Reaction Kinetics

The equilibrium principles described above cannot be applied to the determination of speciation, or changes of speciation, of metal complexes and chelates in biological systems without considering reaction kinetics. Most of the metal ions of interest because of carcinogenic effects establish equilibrium in aqueous solutions with simple chelating and complexing ligands at moderate or rapid rates. Thus most aquo ions would be converted rapidly to complexes or chelates such as acetates, citrates, glycinates, etc., and equilibria would be established in fractions of a minute or less. The main exceptions to this generalization are the trivalent ions, Cr^{3+}, Co^{3+}, and Rh^{3+}. Simple complexes of these ions may require a few days to reach equilibrium. The theoretical basis for this behavior was first described by Taube (*42*).

For the more labile metal ions, combination with multidentate ligands to form complexes containing relatively large numbers of chelate rings may occur very slowly. Rates of substitution may be greatly decreased by steric effects, as in macrocyclic, cryptate, and ionophore complexes of the transition metal ions. In addition, for metal chelates having large numbers of chelate rings, the ligands must "unwrap" from around the metal ion before the latter may be transferred to another chelating ligand. The kinetics of such processes, and the way ligand-ligand metal ion transfer may be catalyzed by additional weak ligands, have been described by Margerum (*43*). An interesting example of this type of catalysis is the acceleration by nitrilotriacetic acid (NTA) of the transfer of iron(III) from transferrin to desferriferrioxamine. Metal chelates of highly multidentate ligands that are rigid and cannot unfold represent the extreme in slow substitution rates. Thus the porphyrin chelates of otherwise reasonably labile transition metal ions are kinetically extremely stable and their rates of

Table 8. Iron (III) affinities of chelating agents in terms of standard and effective stability constants.[a]

No.	Name	Structure	Formula of chelate	n	log β_n	Log K_{eff}
1	2,3-Dihydroxynaphthalene-6-sulfonic acid, H_3L		FeL_3^{6-}	3	44.2	18.20
2	Salicylic acid, H_2L		FeL_3^{3-}	3	35.3	8.01
3	8-Hydroxyquinoline, HL		FeL_3	3	36.9	20.9
4	1,8-Dihydroxynaphthalene-3,6-sulfonic acid, H_4L		FeL_2^{5-}	2	37.0	20.81
5	3-Isopropyltropolone, HL		FeL_3	3	32.0	21.77
6	Acetoxyhydroxamic acid, HL	 $CH_3-C(=O)-NH-OH$	FeL_3	3	28.3	13.10
7	Nitrilotriacetic acid, H_3L		FeL_2^{3-}	2	24.3	8.17
8	Ethylenediaminetetraacetic acid, H_4L		FeL^-	1	25.0	8.10
9	Diethylenetriaminepentaacetic acid, H_5L		FeL^{2-}	1	28.0	10.96

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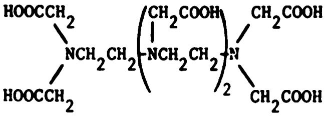
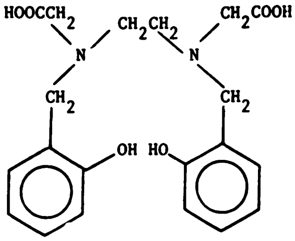
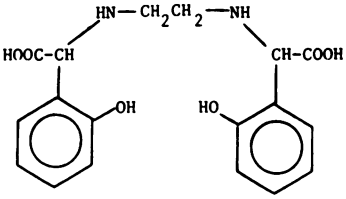
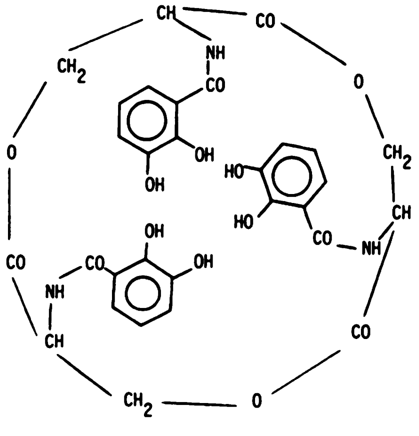
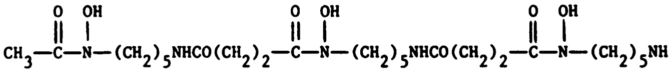
[a] Standard data used in these calculations are: log $\beta_1^{FeOH} = 11.09$; log $\beta_2^{FeOH} = 21.96$; $-\log K_{sp}$ $Fe(OH)_3 = 10^{41}$; log $\beta_4^{FeOH} = 24.4$; $-\log K_w = 13.795$; pH = 7.40.

Table 8. (continued)

No.	Name	Structure	Formula of chelate	n	log β_n	Log K_{eff}
10	Triethylenetetraminehexa-acetic acid, H_6L		FeL^{3-}	1	26.8	10.61
11	N,N′-Bis(*o*-hydroxybenzyl)-ethylenediamine-N,N′-diacetic acid, H_4L		FeL^-	1	39.7	20.78
12	Ethylenebis-N,N′-(2-*o*-hydroxy-phenyl)glycine, H_4L		FeL^-	1	33.9	16.20
13	Enterobactin, H_6L		FeL^{3-}	1	52	25
14	Desferrioxamine B, H_3L	 $CH_3-\overset{\overset{O}{\Vert}}{C}-\overset{\overset{OH}{\vert}}{N}-(CH_2)_5NHCO(CH_2)_2-\overset{\overset{O}{\Vert}}{C}-\overset{\overset{OH}{\vert}}{N}-(CH_2)_5NHCO(CH_2)_2-\overset{\overset{O}{\Vert}}{C}-\overset{\overset{OH}{\vert}}{N}-(CH_2)_5NH_2$	FeL	1	30.6	16.34

metal-ion displacement or transfer are generally immeasurably low.

Future Research Needs

It was suggested above that information on the speciation of metal chelates in biological systems is essential for an understanding of their functions and reaction mechanisms. Metal complexes introduced into the body may undergo exchange with one or more of the large number of natural ligands present in physiological systems, so that the reactions observed may not be characteristic of the original species.

An interesting example of this type of chemical behavior of metal chelates in physiological systems is provided by recent experience with the use of gallium(III) citrate and a number of other gallium(III) complexes and chelates as radiopharmaceuticals. It seems that, regardless of the nature of the gallium compound employed, the patterns of adsorption of radioactive gallium on tumors remained very similar. Eventually it was realized that all moderately stable and weak gallium(III) complexes were converted to the more stable transferrin chelate. Thus the patterns observed turned out to be characteristic of the gallium(III) chelate of transferrin, which has hard phenolate donor groups that form very stable chelates of other trivalent ions as well as of iron(III). For a gallium(III) chelate to retain its integrity in physiological systems, it must be administered in the form a chelate which is significantly more stable than the gallium(III) chelate of transferrin or any other trivalent metal ion receptors that may be present. It seems, therefore, that the study of metal ion reactions in biological systems requires prior information on the stabilities of chelates formed with any synthetic and naturally-occurring ligands that may be present, and that such information is essential to the understanding of the mechanisms of action of metal compounds in the body.

As recently pointed out by Furst (*2*), the carcinogenicity of metal compounds seems to be related to metal-nucleic acid interactions, which may profoundly influence the replication process. Certainly more information is needed concerning the selectivity of binding of metal ions to the bases and phosphate oxygens of DNA.

In nucleic acids the donor groups available for metal ion coordination are the phosphate oxygens of the ribose phosphate backbone, the oxygen and nitrogen atoms of the bases, and to a considerably lesser extent the ribose hydroxyl groups. The donor groups of the bases have affinity for the more electronegative transition metal ions, while more basic metal ions coordinate with the phosphate oxygens. The latter are normally neutralized and coordinated by magnesium ions. The binding of toxic metal ions by nucleic acids either through displacement of Mg^{2+} ions, or by combination with the heterocyclic nitrogen bases, may alter their conformation and structure and lead to impaired function.

DNA polymerase involved in replication requires the interaction of proteins with the DNA template, and Mg^{2+} ions are essential in this process. The substitution of unnatural metal ions such as Be^{2+} for Mg^{2+} results in serious errors in replication. DNA polymerase is bound to DNA by Zn^{2+} ions, while Mg^{2+} binds deoxynucleotide triphosphates to the enzyme. The activating metal ions for RNA polymerase systems are activated by Mg^{2+} and Mn^{2+}, and inhibited by a considerable number of other metal ions. Both replication and transcription require Mn^{2+} and Mg^{2+} for activation, which involves the unwinding of double-stranded DNA and subsequent rewinding. The displacement of these activating metal ions by other metal ions would be expected to strongly interfere with the replication process. The much higher affinity of Be^{2+} over Mg^{2+} for the phosphate oxygens, for example, could lead to irreversible changes in structure and function.

Since most essential trace metals are carcinogenic, it seem probable that the observed carcinogenic properties occur when there is an imbalance, or a large excess, of a metal ion over the concentration required for normal function, or over the amounts that can be handled by the protective mechanisms available in the body. On the basis of these tentative concepts it becomes obvious that the testing of these theories and the development of an understanding of metal carcinogenesis will require a much higher level of knowledge than now exists on the speciation of metals in the various compartments of the body and the changes of speciation that occur when metal ions migrate from one compartment to another, as the result of changes of conditions and of changes in the natural ligands available. Only with such information will it be possible to carry out controlled experiments on carcinogenic metal compounds under conditions such that the nature of the activating metal species will be known.

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